Effect of Nitrous Acid on Lung Function in Asthmatics: A Chamber Study

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Nitrous acid, a component of photochemical smog and a common indoor air pollutant, may reach levels of 100 ppb where gas stoves and unvented portable kerosene heaters are used. Nitrous acid is a primary product of combustion and may also be a secondary product by reaction of nitrogen dioxide with water. Because the usual assays for nitrogen dioxide measure several oxides of nitrogen (including nitrous acid) together, previous studies of indoor nitrogen dioxide may have included exposure to and health effects of nitrous acid. To assess the respiratory effects of nitrous acid exposure alone, we carried out a double-blinded crossover chamber exposure study with 11 mildly asthmatic adult subjects. Each underwent 3-hr exposures to 650 ppb nitrous acid and to filtered room air with three 20-min periods of moderate cycle exercise. Symptoms, respiratory parameters during exercise, and spirometry after exercise were measured. A statistically significant decrease in forced vital capacity was seen on days when subjects were exposed to nitrous acid. This effect was most marked at 25 min and 85 min after exposure began. Aggregate respiratory and mucous membrane symptoms were also significantly higher with nitrous acid. We conclude that this concentration and duration of exposure to nitrous acid alters lung mechanics slightly, does not induce significant airflow obstruction, and produces mild irritant symptoms in asthmatics. *Key words*: asthma, indoor air pollution, nitrogen dioxide, nitrous acid. *Environ Health Perspect* 103:372–375 (1995)

Nitrous acid (HNO_2) is the major gas-phase acid in environmental tobacco smoke (*1*) and in its vapor phase is found in automobile emissions. Although outdoor ambient concentrations are less than those of sulfuric acid (H_2SO_4) and nitric acid (HNO_3), up to 8 ppb HNO_2 has been measured in ambient air in California during an air pollution episode (*2*). In homes with combustion sources, elevated HNO_2 levels may be associated with direct emissions from the source as well as with reactions of emitted NO_2 with water vapor in air. Indoor concentrations of HNO_2 are higher than outdoor concentrations, even when indoor concentrations of NO_2 do not exceed outdoor levels. Peak levels of HNO_2 may exceed 50 ppb and persist for several hours (*3,4*). Nitrous acid may also be a secondary reaction product of NO_2 with water on indoor surfaces and, under experimental conditions, has been found to make up as much as 10% of oxides of nitrogen after an interval of reaction (*5*). Conventional assays of NO_2 measure several oxides of nitrogen together, including HNO_2. For this reason, previous studies of respiratory effects of indoor NO_2 may have included exposures to HNO_2 without independent measurement of exposure and effect (*6*).

Based on *in vitro* studies, it has been postulated that at environmental concentrations HNO_2 is formed within the respiratory system predominantly by hydrogen abstraction (*7*), with subsequent conversion of HNO_2, at physiologic pH, to H^+, and NO_2^- (*7*). It has been proposed that HNO_2 formed in this way may contribute to the bronchoconstricting effects of NO_2 seen in normal subjects and asthmatics. Studies of the direct effects of HNO_2 on the human respiratory system are thus of interest because exposures may occur from primary indoor and outdoor sources or from reaction products of NO_2 formed within the human respiratory system. A need for more information on the health effects of HNO_2 has recently been identified (*8*).

We performed a chamber exposure study to determine whether there is an effect on respiratory symptoms or lung mechanics in a group of patients (mild asthmatics) who have been demonstrated in some but not all studies to be sensitive to other acid species (*9–12*). We used a concentration of HNO_2 higher than that usually measured in homes with unvented combustion sources (*4*), but the duration of exposure was shorter than may occur in such homes.

Methods

Subjects. The protocol was approved by the Yale University School of Medicine Human Investigations Committee, and all subjects gave informed consent to participate. The 11 subjects were recruited by advertisements and were selected using the following inclusion criteria: age between 18 and 40 years, nonsmoking, and in good general health other than mild asthma (as defined by a physician's diagnosis with typical symptoms and occasional but not regular use of bronchodilator medications). In addition to these criteria, all subjects had baseline forced expiratory volume in 1 sec (FEV_1) and forced vital capacity (FVC) within the normal range for age, sex, and height (*13*) and had methacholine reactivity within the asthmatic range [a provocative concentration (PC) of methacholine less than 8 mg/mL causing a 20% fall in FEV_1 excepting subject 2, whose PC_{20} was 26 mg/mL]. Exclusion criteria included regular or current use of bronchodilator medications, current active asthma symptoms, presence of wheezing on physical exam, or inability to comfortably perform moderate cycle exercise for 20 min. Subjects did not need or use asthma medications during the days before or during the chamber studies. Once accepted into the study, subjects had a training session with spirometry and a cycle ergometer exercise session during which a workload tolerable for 20 min was determined.

Protocol. Each subject underwent two 3-hr intermittent exercise chamber exposures which differed only in that one was conducted with continuous HNO_2 exposure at a target level of 700 ppm, while the other was conducted with filtered, clean air. Air temperature was maintained at 18°C during exposure to provide a comfortable ambient environment for sustained moderate exercise. The 3-hr chamber exercise periods were performed in a balanced, randomized double-blinded crossover design, so that six subjects were exposed to HNO_2 on their first test day and five to clean air. Exposures were separated by a 1- to 2-week washout period. Subjects and investigators (who entered the chamber with subjects to perform measurements of exercise responses and resting lung mechanics) were blinded as to whether exposure was to HNO_2 or to clean air. The effectiveness of the blinding procedure was assessed by asking subjects and investigators to indicate, at the end of each exposure session, whether they believed exposure had been to HNO_2 or clean air.

During each 3-hr exposure, subjects completed a baseline symptom questionnaire and spirometry immediately on entering the chamber, and then 20 min of cycle ergometer exercise at their predetermined constant workload at the start of each hour. Heart rate (HR) (Polar Electro Inc., Heartland, Wisconsin), minute ventilation (V_E), and tidal volume (V_T) (5410 volume meter, Ohmeda, Englewood

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Colorado, calibrated with a Tissot spirometer) were measured at 5, 10, and 15 min of each exercise period. Immediately after exercise, subjects completed the symptom questionnaire, and then performed spirometry within the chamber 5 min later (Eagle II Stead Wells survey spirometer, W.E. Collins Inc., Braintree, Massachusetts). American Thoracic Society criteria for standardization of spirometry were applied (*14*). Subjects then rested in a seated position within the chamber until the next exercise session. A final spirometry and questionnaire were completed at the end of 180 min, just before leaving the chamber.

Symptoms. Subjects completed the same symptom questionnaire five times over each exposure session. They rated each symptom by placing a mark on a 10-cm continuous line representing a score of "absent" through "the most severe ever experienced." Four respiratory symptoms (shortness of breath, wheeze, cough, chest tightness), six sensory irritant questions (skin irritation, eye irritation, eye tearing, throat irritation, nasal stuffiness, nasal dryness), and one negative control question (headache) were included.

Generation of HNO_2. Nitrous acid was generated by a reaction of sodium nitrite with sulfuric acid using the method of Taira and Kanda (*15*). A solution of 0.08 M sulfuric acid and a solution of 0.06 M sodium nitrite were prepared with distilled deionized water. A peristaltic pump added each of the solutions at 2 ml/min onto a circular piece of fritted glass, which was located near the base of the reaction chamber. Ambient air was filtered through a system of Purafil (potassium permanganate-coated aluminum) and activated charcoal and passed into the reaction chamber below the glass frit at 20 L/min. The cleaned air passed through the glass frit and bubbled through the reagent mixture, removing HNO_2 from the solution. The mixture was passed through a condensing chamber to remove excess water vapor. This HNO_2-containing gas was then fed into the exposure chamber. Excess reagent was removed from the reaction chamber by three tubes located 8 mm above the reagent inlet, and connected to a vacuum flask and a vacuum pump.

Measurement of HNO_2. We monitored the nitrous acid concentration in the chamber air by a continuous method using a chemiluminescent NO_x analyzer with a system of filters and a valve. Two filter packs were set up in parallel to the NO_x analyzer inlet, with a valve switching from one filter pack to the other every 2 min. One filter pack contained a glass-fiber filter coated with sodium carbonate and glycerol, and the other contained an uncoated filter. The coated filter removed the HNO_2 from the air passing through it, while allowing the NO and NO_2 to pass through. The uncoated filter did not remove any of these gases. The NO_x analyzer measured HNO_2 as the difference in signal with and without the coated filter.

We also used a noncontinuous method of measuring HNO_2 employing the Harvard EPA Annular Denuder System (*16*) with two denuders in series sampling at 4 L/min. In this system the sample air passed through the space between concentric glass tubes (the annular denuder), which was coated with sodium carbonate and glycerol. Nitrous acid diffused to the coated walls and was trapped, while nonacidic gases passed through uncollected. The denuders were extracted with ultra-pure water after the sampling period. The concentration of nitrite ion in the denuder extract was measured by ion chromatography to allow determination of the total amount of nitrous acid that passed through the denuder system. The chamber HNO_2 concentration was then calculated from this value. The chamber size was 18 m^3 (624 ft^3), and the ventilation rate averaged approximately 30 ft^3/min, or three air changes per hour.

Statistical analysis. To ensure comparable baseline pulmonary function at the start of each session, we compared the two pre-exercise spirometry readings using a paired *t*-test. The effects of HNO_2 exposure, time, and their interactive influence upon exercise and spirometry measurements were evaluated with repeated measures analysis of variance. We analyzed spirometry results with actual values and expressed them as percent predicted (*13*). Symptom scores for each symptom of the four questionnaires completed 20, 80, 140, and 180 min after entering the chamber were combined as a mean score, and results from the exposure day compared with clean air by the Wilcoxon signed-rank sum test (*17*). For all analyses, a two-sided significance level was chosen at p <0.05. Due to equipment failure, minute ventilation, tidal volume, and respiratory rate were not obtained for one subject at a single time point. For purposes of statistical analysis, this subject's data were deleted at all time points. Data were analyzed using Systat statistical software (Systat Inc., Evanston, Illinois).

Results

All 11 subjects selected for inclusion in the study completed the protocol. Subject characteristics are listed in Table 1, along with the ergometer workload and minute ventilation established during the practice session. The mean HNO_2 concentration and standard deviation within the chamber on exposure days was 648 ± 41 ppb. (Measurements of HNO_2 were not made in the homes of subjects).

Subjects were successfully blinded as to exposure conditions, in that they correctly identified the chamber exposure conditions of only 27% of the sessions. The investigators were also successfully blinded, identifying exposure correctly in 60% of the sessions (which was not significantly different from an expected 50%; 95% CI, 39–81%).

Results of serial spirometry during HNO_2 control and exposure days are shown in Figure 1. Baseline values were not different at the beginning of control and exposure days, but there was a statistically significant decrease in FVC during HNO_2 exposure which was most marked at 25 min after the beginning of exposure and persisted throughout the 3-hr exposure (p = 0.017 when vital capacity expressed as absolute value; p = 0.020 when vital capacity expressed as percent predicted). The

Table 1. Characteristics of mildly asthmatic subjects and workload set during exercise

Subject no.	Age (years)	Sex	Height	FEV_1 (%)	FVC (%)[a]	PC_{20}[b]	Work (kpm/min)	Minute ventilation (L/min)
1	28	F	160	3.10 (104)	3.60 (97)	0.6	360	35
2	24	F	169	3.79 (110)	4.64 (108)	26	180	23
3	23	M	173	3.71 (87)	4.53 (86)	4	540	34
4	38	F	171	3.35 (109)	4.25 (108)	8	270	32
5	26	F	156	2.79 (98)	3.09 (87)	2	360	31
6	28	M	172	3.34 (81)'	4.73 (92)	1	450	48
7	27	F	157	2.94 (101)	3.59 (99)	2	450	33
8	35	M	164	3.92 (109)	4.65 (103)	2	630	39
9	39	F	164	2.91 (101)	3.16 (86)	0.2	540	25
10	30	F	157	2.60 (92)	3.26 (92)	0.7	360	36
11	18	M	167	3.75 (88)	4.00 (79)	3	540	30
Mean	29		165	3.29 (98)	3.95 (94)	4.5	425	33
SD	7		6	0.45 (9)	0.64 (10)	7.5	134	7
SE	2		2	0.14 (3)	0.19 (3)	2.25	40	10

Abbreviations: FEV_1, forced expiratory volume in 1 sec; FVC, forced vital capacity; kpm, kilopond-meters.
[a]Predicted normal values from Morris et al. (*13*).
[b]PC_{20} FEV_1 methacholine: the provocative concentration of inhaled methacholine chloride, causing a 20% decline from the baseline FEV_1.

difference between control and exposure FVC was small: at 25 min the mean difference was 108 mL, representing a mean difference of approximately 3% between exposure and control conditions. Mean values of FEV_1 were also not different at the beginning of control or exposure sessions, and no effect of HNO_2 exposure was seen on expiratory airflow at the beginning of expiration (FEV_1) or during midflow (MMEF, maximal mid-expiratory flow rate). Although the controlled design of this study permitted evaluation of exercise-induced bronchospasm independently of any effect of HNO_2 exposure, little exercise-induced bronchospasm was seen in this group of mild asthmatics. The maximum mean FEV_1 decline from pre-exercise baseline was 32 mL on the control days and 39 mL on the exposure days, representing an approximately 1% post-exercise decline. Because spirometry was performed 5 min after exercise, delayed exercise-induced bronchospasm, which may be maximal more than 5 min after the end of exercise, may not have been detected.

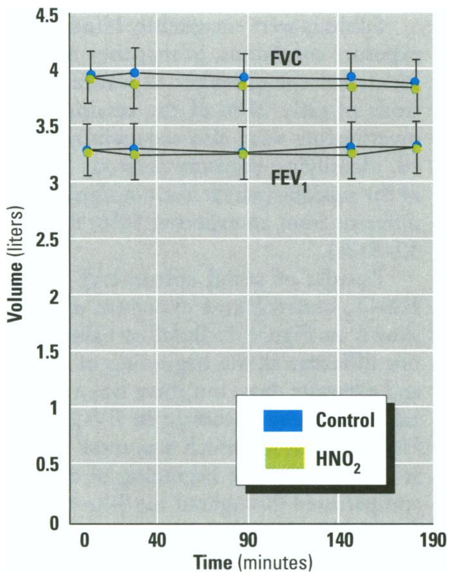

Figure 1. Mean forced vital capacity (FVC) and forced expiratory volume in the first second of expiration (FEV_1) for 11 subjects just before the start of the chamber sessions and at 25, 85, 145, and 180 min of exposure (bars represent SE of the mean). Vital capacity on HNO_2 exposure days was significantly lower than on control days.

Serial measures of heart rate, minute ventilation, tidal volume, and breathing frequency on control and exposure days are shown in Table 2. Comparison showed no statistically significant effects of HNO_2 exposure on these responses to exercise. The comparable values of HR and V_E under the two exposure conditions indicate comparable exercise workloads and cardiovascular and ventilatory responses on control and exposure days.

Mean symptom scores reported during exposure were low under both control and exposure conditions, ranging from 0.0 to 1.0 on a 10-point scale (Table 3). As reflected in the successful blinding, the subjects were uncertain as to whether exposure sessions were to HNO_2 or clean air. However, the aggregate score of all 10 test symptoms was higher on the HNO_2 than control days. This difference was small but statistically significant ($p = 0.038$). The difference between the mean score on exposure and control days for the negative control symptom (headache) was lower than for seven other symptoms and identical to that for wheeze, cough, and nasal stuffiness.

Discussion

Nitrous acid at 650 ppb over a 3-hr exposure period is a weak sensory irritant, as demonstrated by the failure of subjects to distinguish exposure from control days. Nonetheless, a physiologic effect of exposure was detectable with this concentration and duration, as seen in the alteration of static lung mechanics in these mildly asthmatic subjects. The small, statistically significant effect on FVC in the absence of an effect on FEV_1 or MMEF suggests that the exposure–duration combination used in this study is above, but not far, from the threshold for effects on lung mechanics. The primary response at this dose, a reduction in vital capacity, may be due either to inhibition of maximal inspiratory effort, a reduction in respiratory system compliance, or closure of airways at higher lung volumes. However, because of the solubility of HNO_2 in airway mucosal water and the relatively low concentration tested, it is likely that most of the vapor is absorbed in the respiratory mucosa before reaching terminal bronchioles and alveoli. For this reason, we speculate that the mechanism for this effect is inhibition of maximal inspiration due to effects on sensory afferent nerves. This mechanism has been demonstrated for ozone, a potent respiratory irritant, at 500 ppb (*18*). However, because ozone is an aqueous, insoluble gas which is poorly absorbed in the upper airways and HNO_2 would be expected to be well absorbed in the upper airways, these data raise the possibility that the effect seen was due to stimulation of upper airway receptors, having the effect of inhibiting maximal inspiration.

Asthmatic subjects were chosen for this study as a potentially more sensitive clinical group. Because only asthmatics were studied, we do not know whether nonasthmatic subjects are less susceptible, or more severe asthmatics are more susceptible, to this concentration and duration of exposure.

Nitrous acid is of interest as an environmental exposure due to its presence in emissions from automobiles, natural gas and kerosene-burning appliances, and environmental tobacco smoke. It may also be a reaction product of inhaled NO or NO_2 within the respiratory system. Still, little information is available on respiratory system effects of nitrous acid alone. An *in vitro* study has demonstrated that HNO_2 is capable of functionally inactivating human plasma α-1 proteinase inhibitor in a 0.05 M sodium acetate buffer solution when incubated for 15 min at 25°C, pH 4.0. (*19*). Two studies have suggested, on the basis of *in vitro* simulations and studies in isolated perfused rat lungs, that inhaled NO_2 undergoes nonsaturable uptake or transformation in the lung, forming low molecular weight soluble reaction products, the predominant one being HNO_2. Using cyclo-hexane to simulate lung lipid with *in*

Table 2. Exercise parameters (means ± SEM) during control and HNO_2 exposure days[a]

		Time (min)								
		5	10	15	65	70	75	125	130	135
HR	Control	126 ± 4	130 ± 5	135 ± 5	125 ± 5	126 ± 4	132 ± 5	128 ± 5	133 ± 5	135 ± 6
	HNO_2	131 ± 4	133 ± 5	135 ± 4	127 ± 4	132 ± 4	136 ± 4	132 ± 5	134 ± 3	136 ± 4
V_E (L/min)	Control	33 ± 2	34 ± 2	34 ± 2	31 ± 2	32 ± 2	33 ± 2	32 ± 2	34 ± 2	35 ± 2
	HNO_2	33 ± 2	35 ± 3	34 ± 2	31 ± 2	32 ± 2	34 ± 2	35 ± 2	36 ± 2	35 ± 2
V_t (L)	Control	1.70 ± 0.18	1.58 ± 0.12	1.80 ± 0.20	1.73 ± 0.18	1.65 ± 0.17	1.64 ± 0.14	1.63 ± 0.13	1.70 ± 0.16	1.74 ± 0.16
	HNO_2	1.69 ± 0.16	1.64 ± 0.15	1.62 ± 0.13	1.67 ± 0.13	1.53 ± 0.10	1.61 ± 0.12	1.68 ± 0.14	1.78 ± 0.12	1.61 ± 0.13
f	Control	20 ± 2	22 ± 1	21 ± 1	19 ± 1	21 ± 2	22 ± 2	23 ± 3	21 ± 2	21 ± 1
(breaths/min)	HNO_2	21 ± 2	22 ± 2	22 ± 2	20 ± 1	23 ± 1	22 ± 1	22 ± 1	21 ± 2	23 ± 2

Abbreviations: HR, heart rate; V_E minute ventilation, V_t tidal volume, f, breathing rate.
[a]There were no statistically significant differences between control and HNO_2 measurements for any parameter.

Table 3. Cumulative symptom responses (10-point scale) for all subjects with control air and HNO_2 chamber exposures[a]

Question	Exposure status	Mean value
Shortness of breath	Air	0.625
	HNO_2	1.075
Wheeze	Air	0.1
	HNO_2	0.125
Cough	Air	0.25
	HNO_2	0.275
Chest tightness	Air	0.25
	HNO_2	0.325
Skin irritation	Air	0.25
	HNO_2	0.375
Eye irritation	Air	0.60
	HNO_2	0.65
Eye tearing	Air	0.225
	HNO_2	0.225
Throat irritation	Air	0.150
	HNO_2	0.4
Nasal stuffiness	Air	0.625
	HNO_2	0.75
Nasal dryness	Air	0.70
	HNO_2	0.725

[a]The aggregate score of all 10 test symptoms was significantly higher on the HNO_2 exposure than the control days. Each symptom was assessed just before, during, and immediately after exposure. (Headache was included in the questionnaire as a negative control symptom and was not included in this analysis.)

vitro conditions simulating low exposure to nitrogen dioxide (less than 100 ppm), Pryor and Lightsey (*20*), proposed that the conversion of NO_2 to HNO_2 is initiated according to the following reaction:

$$NO_2 + -HC = CH-CH_2- \rightarrow HNO_2 + -CH-CH-CH-$$

which is similar to the mechanism for formation of HNO_2 from NO_2 postulated on the basis of experimental observations in airways (*7,21*).

The rate of tissue absorption of a vapor as it is inhaled in the respiratory system is determined by its concentration, the solubility of the vapor in water, and the rate of airflow. The effective solubility (Henry) coefficient of HNO_2 is close to that of sulfur dioxide at physiologic pH (*22*), and increases with increasing pH over the range from 2 to 6 (*23*). Comparisons of the respiratory effects of acidic gases and aerosols of varying compositions indicate that the hydrogen ion content of the substance is one of the important determinants of the effect on airways. A study of the effect of inhaled acid aerosols in asthmatics has suggested that titratable acidity, as well as the specific chemical composition and pH, are important determinants of the potency of acid in producing effects on lung mechanics (*24*).

Asthmatics were selected for the present study because of previously demonstrated susceptibility to airway effects of inhaled acidic aerosol (*9*). Increased sensitivity of asthmatic subjects to acidic aerosols has not been seen in all such studies (*11,12*). The duration of the exposure in this study was three times as long as the exposures reported by Avol et al. (*11*) and Aris et al. (*12*), and may account for the significant effect on lung function seen in the present study. Bronchoconstriction was not seen at this dose and duration, even though forced vital capacity was reduced. Further study will be needed to determine whether asthmatics differ in their susceptibility to the effects of vapor-phase HNO_2 from nonasthmatics and whether airway constriction is seen at dose–duration combinations higher than those used in this study.

In summary, when exposed for 3 hr with intermittent, moderate exercise to 650 ppb HNO_2, mildly asthmatic subjects experienced a small decrease in FVC which was apparent within 25 min of the onset of exposure. They also reported a slightly higher aggregate rate of respiratory and mucous membrane symptoms, although at this dose they were not able to distinguish exposure from control days. These data suggest that the experimental dose of HNO_2 used is slightly above but very close to the threshold for respiratory effects of HNO_2.

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